

**Amendments to the Specification:**

Please replace the section titled BRIEF DESCRIPTION OF DRAWINGS AND SEQUENCE LISTINGS page 12 line 10 through page 15 line 6 with the following amended paragraphs:

**BRIEF DESCRIPTION OF DRAWINGS AND SEQUENCE LISTINGS**

Fig. 1A, Fig. 1B, Fig. 1C and Fig. 1D is a schematic representation of the method according to the invention compared to prior dimeric immunoglobulin Fc fusion.

~~On the left~~ Fig 1A is a side elevation view and Fig. 1B is a top plan view: Structural characteristics of a homodimeric soluble sTNF RII receptor-Fc fusion, such as Amgen's Enbrel, in either ligand-free or -bound form as indicated.

Domains labeled in green denote soluble TNF-RII. Note that the Fc (labeled in light blue with inter-chain disulfide bonds in red) fusion protein is dimeric in structure. Given its 2-fold symmetry, the dimeric Fc fusion protein is bivalent and thus theoretically does not have the optimal conformation to bind to a homotrimeric ligand, such as TNF- $\alpha$  (labeled in brown), which has a 3-fold symmetry.

~~On the right~~ Fig 1C is a side elevation view and Fig. 1D is a top plan view: Structural characteristics of a trimeric soluble sTNF RII receptor-C-propeptide fusion.

Given its 3-fold symmetry, a sTNF RII-Trimer fusion protein is trivalent in nature, thus can perfectly dock to its trimeric ligand TNF- $\alpha$ . ~~[[□□□]]~~ C-propeptide of collagen

capable of self trimerization is labeled in dark blue with inter-chain disulfide bonds labeled in red.

**Fig. 2A and Fig. 2B** illustrate[[s]] the structures of pTRIMER plasmid vectors for creating secreted trimeric fusion proteins. Any soluble receptor- or biological active polypeptide-encoding cDNAs can be cloned into the unique Hind III or Bgl II sites to allow in-frame fusion at the C-termini to the  $\alpha$  (I) collagen containing C-propeptide sequence for trimerization. **Fig. 2A:** The pTRIMER(T0) construct contains part of the glycine-repeats (GXY)<sub>n</sub> upstream of the C-propeptide; **Fig. 2B:** whereas the pTRIMER(T2) contains only the C-propeptide domain with a mutated BMP-1 protease recognition site.

**Fig. 3A and Fig. 3B** illustrate[[s]] the expression and secretion of disulfide bond-linked trimeric collagen fusion proteins.

**Fig. 3A.** Western blot analysis of the trimerization of human placental alkaline phosphatase (AP) when fused to the C-propeptides of  $\alpha$ (I) collagen. The expression vectors encoding either AP alone or AP-C-propeptide fusions in pTRIMER vectors were transiently transfected into HEK293T cells. Forty-eight hours later, the conditioned media (20  $\mu$ L) of each transfected cells as indicated were boiled for 5 minutes in equal volume of 2X SDS sample buffer either with or without reducing agent (mercaptoethanol), separated on a 10% SDS-PAGE and analyzed by Western blot using a polyclonal antibody to AP (GenHunter Corporation). Note the secreted 67 kDa AP alone does not form intermolecular disulfide bonds, whereas the secreted AP-T0 and AP-T2 fusions both are assembled efficiently into disulfide bond linked trimers.

**Fig. 3B.** Western blot analysis of the trimerization of soluble human TNF-RII when fused to the C-propeptides of  $\alpha(I)$  collagen. The expression vectors encoding either the AP—C-propeptide fusion (T2) (as a negative control for antibody specificity), or human soluble TNF-RII-C-propeptide fusions as indicated in pTRIMER vectors were transiently transfected into HEK293T cells. Forty-eight hours later, the conditioned media (20  $\mu$ L) of each non-transfected and transfected cells as indicated were boiled for 5 minutes in equal volume of 2X SDS sample buffer either with or without reducing agent (mercaptoethanol), separated on a 10% SDS-PAGE and analyzed by Western blot using a monoclonal antibody to human TNF-RII (clone 226, R & D Systems, Inc.). Note the monoclonal antibody can only recognize the secreted TNF-RII with disulfide bonds. Both the soluble TNF-RII-T0 and TNF-RII-T2 fusions are assembled efficiently into disulfide bond linked trimers.

**Fig.4 and Fig. 5.** illustrate[[s]] the bioassays showing the potent neutralizing activity of the trimeric soluble human TNF-RII-C-propeptide fusion protein against human TNF- $\alpha$  mediated apoptosis.

**Fig. 4[[A]].** The TNF- $\alpha$  sensitive WEHI-13VAR cells (ATCC) were resuspended at 1 million cells/mL in RPMI medium containing 10% FBS. 100  $\mu$ L of the cell suspension was plated into each well in a 96-well microtiter plate. Actinomycin D was added to each well at 500 ng/mL concentration followed by human TNF- $\alpha$  at 500 pg/ml (R & D Systems) in the presence or absence of trimeric soluble human TNF-RII-T2 as indicated. As a negative control, the trimeric AP-T2 was added in place of TNF-RII-T2. After 16 hours of incubation in a tissue culture incubator, the viability of cells was examined using either an inverted microscope at 20X magnification or cell viability indicator dye, Alamar

Blue (BioSource, Inc.) added to 10% (v/v) to each well. The live cells are able to turn the dye color from blue to pink. Note that the trimeric soluble human TNF-RII-T2 exhibits a potent neutralizing activity against TNF- $\alpha$ . ~~□□□□□~~ protects the cells from TNF- $\alpha$  mediated ~~apoptosis~~ apoptosis.

**Fig. 5[[B]].** Quantitative analysis of the neutralizing activity of trimeric soluble human TNF-RII-T2 against human TNF- $\alpha$ . The experiment was carried out as Fig. 4A. Two hours after adding the Alamar Blue dye, the culture medium as indicted from each well was analyzed at OD575. The readings were normalized against wells with either no TNF- $\alpha$  (100% viability) added or with TNF- $\alpha$  without neutralizing agent (0% viability) added.